APPENDIX A

Talkington, Jeffrey C.

From: Baback Gharizadeh [baback@stanford.edu]
Sent: Monday, January 08, 2007 10:12 AM
To: Talkington, Jeffrey C.
Subject: Fwd: FW: Questions

Good morning Jeff,

Attached you find a response from the corresponding author of the Rady et al. The have not used any multiple sequencing primers. They have used only specific single primer for each Sanger sequencing reaction.

Please let me know if there is anything I can do to help the process.

Best regards
Baback

>X-Sieve: CMU Sieve 2.3
>Delivered-To: baback@stanford.edu
>Subject: FW: Questions
>Date: Mon, 8 Jan 2007 11:55:47 -0600
>Thread-Topic: Questions
>Thread-Index: Accx/PizfW+3hY/6SkK7AyYTYVkWtgAfI0AuADQWrQAAAQuMkA==
>From: "Stephen Tyring" <styring@ccstexas.com>
>To: <baback@stanford.edu>
>
>
>
>-----Original Message-----
>From: Rady, Peter [mailto:Peter.Rady@uth.tmc.edu]
>Sent: Monday, January 08, 2007 11:40 AM
>To: Stephen Tyring
>Subject: RE: Questions
>
>Suggestions to the response:
>1./ We did not use multiple type-specific sequencing primers as a pool.
>We used single single type-specific primer for each reaction.
>2./ We used single type-specific primer for each reaction in figure 4.
>
>The PCR conditions we gave in the article need to be optimized on the
>PCR instruments used by Dr Gharizadeh.
>
>
>_____
>
>From: Baback Gharizadeh [mailto:baback@stanford.edu]
>Sent: Sat 1/6/2007 7:41 PM
>To: Stephen Tyring
>Subject: Questions
>
>
>Dear Dr Stephen Tyring,
>
>My name is Baback Gharizadeh and I have read your paper from 1995 from
>Journal of Virological Methods. I have a couple of questions regarding
>your paper that are unclear to me. I would really appreciate your time.
>
>Rady PL, Arany I, Hughes TK, Tyring SK.
>Type-specific primer-mediated direct sequencing of consensus
>primer-generated PCR amplicons of human papilloma viruses: a new
>approach for the simultaneous detection of multiple viral type
>infections.
>J Virol Methods. 1995 Jun;53(2-3):245-54.

>
>1. Have you used multiple type-specific sequencing primers (as a pool)
>in each sequencing react for detection of HPV? Or have you used single
>type-specific sequencing primers separately where the amplicons are
>mixed (multiple co-infections)? Figure 2 and 3 show sequence Sanger
>results. Are they sequenced by a pool of multiple sequencing primers or
>single type specific primer for each reaction?
>
>2. If you have used multiple type-specific sequencing primers (in a
>pool) for each sequencing reaction, how do you differentiate different
>HPV genotypes present in an amplicon? As in figure 4 A you can see HPV
>16 and 33 very clearly. Have you used single specific primer for each
>reaction in figure 4? If we have HPV 16 and 33 in one sample and we use
>the sequencing primers in a pool for a Sanger sequencing reaction, we
>get non-interpretable sequence results. Could it be that you have used
>single type-specific primer for each reaction in figure 4?
>
>I hereby thank you in advance for taking your time to reply to my
>questions.
>
>Many thanks
>Sincerely
>Baback Gharizadeh
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>--
>_____
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